

Supplementary Materials for **Essential Role of DAP12 Signaling in Macrophage Programming into a Fusion-Competent State**

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Published 28 October 2008, *Sci. Signal.* **1**, ra11 (2008)
DOI: 10.1126/scisignal.1159665

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Supplementary Materials

		MFI CD11b
ResMΦ	WT	324 ± 36
	DAP12-KI	329 ± 13
SpleenMΦ	WT	36 ± 2
	DAP12-KI	36 ± 4
ThioMΦ	WT	48 ± 4
	DAP12-KI	57 ± 2

Table S1. Abundance of surface CD11b is normal in DAP12-KI macrophages. CD11b abundance was measured as mean fluorescence intensity (MFI) in F4/80 and CD11b double-positive cells (F4/80+/CD11b+) from the peritoneal cavity (resMΦ), the spleen (SpleenMΦ), and from the peritoneal cavity 4 days after thioglycollate injection (ThioMΦ) by FACS analysis (n = 3). Results were confirmed in 2 independent experiments.

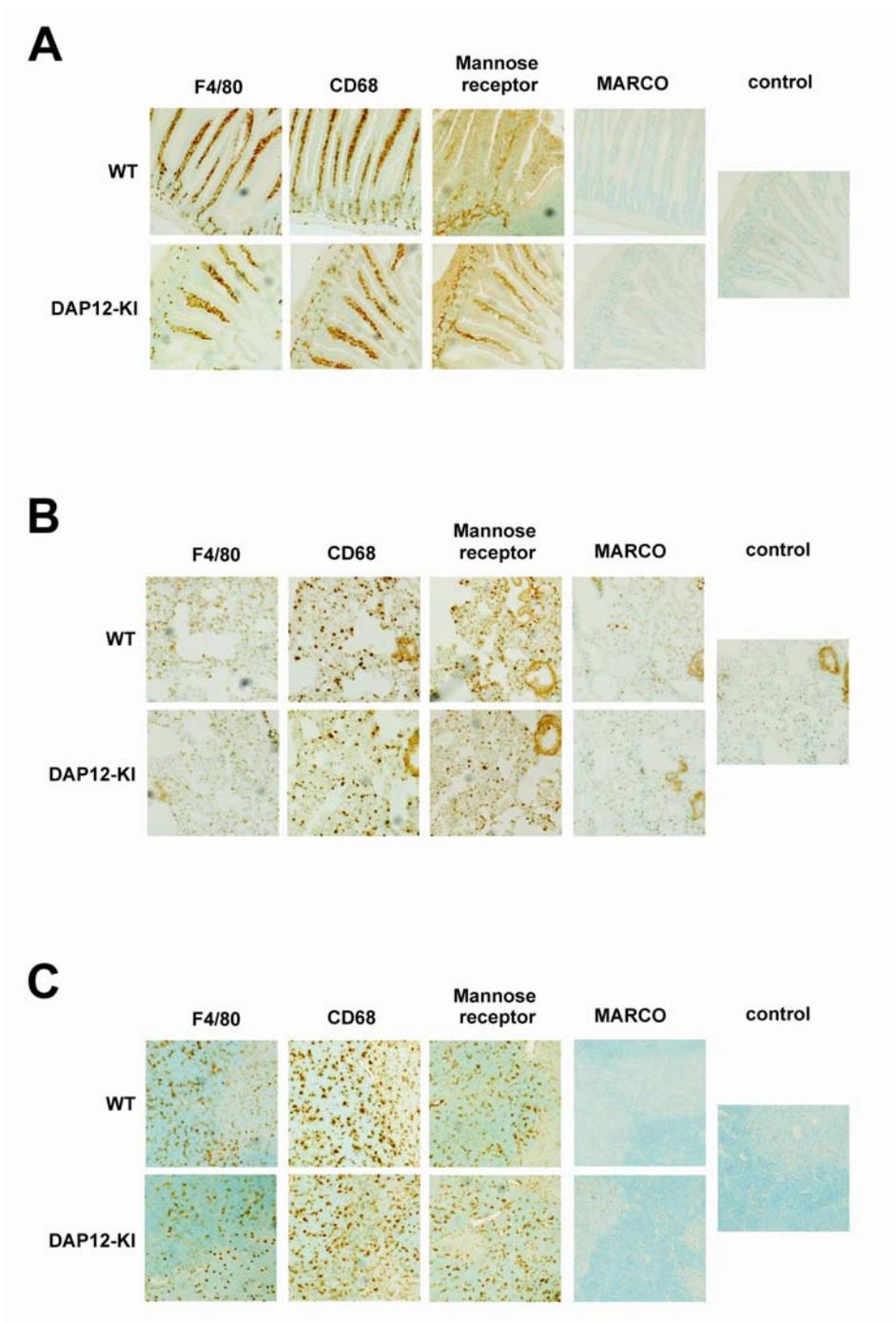


Fig. S1. Macrophage distribution is normal in organs of DAP12-KI mice. Frozen sections of gut (A), lung (B) and thymus (C) from WT and DAP12-KI mice were stained with F4/80, FA11 (CD68), 5D3 (mannose receptor), and ED31 (MARCO); staining was detected with HRP-conjugated secondary antibodies and nuclei were counterstained with methyl green. Control = secondary antibody.

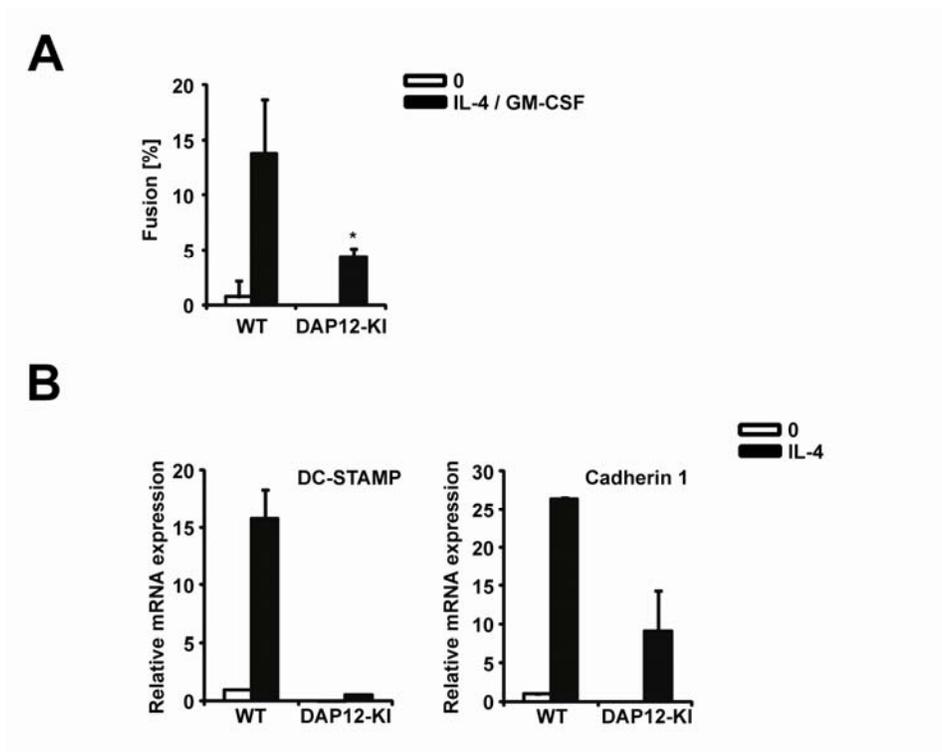


Fig. S2. Macrophage fusion and expression of fusion mediators are reduced in DAP12-KI BMM. (A) BMM from WT and DAP12-KI mice were cultured in the presence or absence of IL-4 and GM-CSF. Macrophage fusion was quantified as the percentage of giant cell nuclei relative to the total number of nuclei. Shown are means \pm s.d. ($n = 3$). * $P \leq 0.05$ (Student T Test, two-tailed). (B) WT and DAP12-KI BMM were cultured in the presence or absence of IL-4 for 24 hrs. RNA was isolated and analyzed by real-time PCR for DC-STAMP and Cadherin 1 expression. Expression was normalized to a housekeeping gene (HPRT), as well as the unstimulated WT control. Shown are means \pm s.d. of duplicate measurements. Results were confirmed in 2 independent experiments.

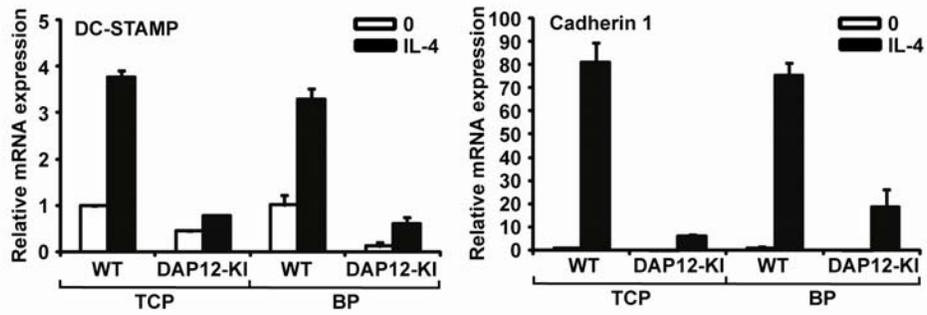


Fig. S3. Reduced expression of fusion mediators in DAP12-KI macrophages on different tissue culture surfaces. WT and DAP12-KI ThioM Φ were cultured in the presence or absence of IL-4 for 24 hrs on tissue culture treated plastic (TCP) or bacteriological plastic (BP). RNA was isolated and analyzed by real-time PCR for DC-STAMP and Cadherin 1 expression. Expression was normalized to a housekeeping gene (HPRT), as well as the unstimulated WT control. Shown are means \pm s.d. of duplicate measurements. Results were confirmed in 2 independent experiments.

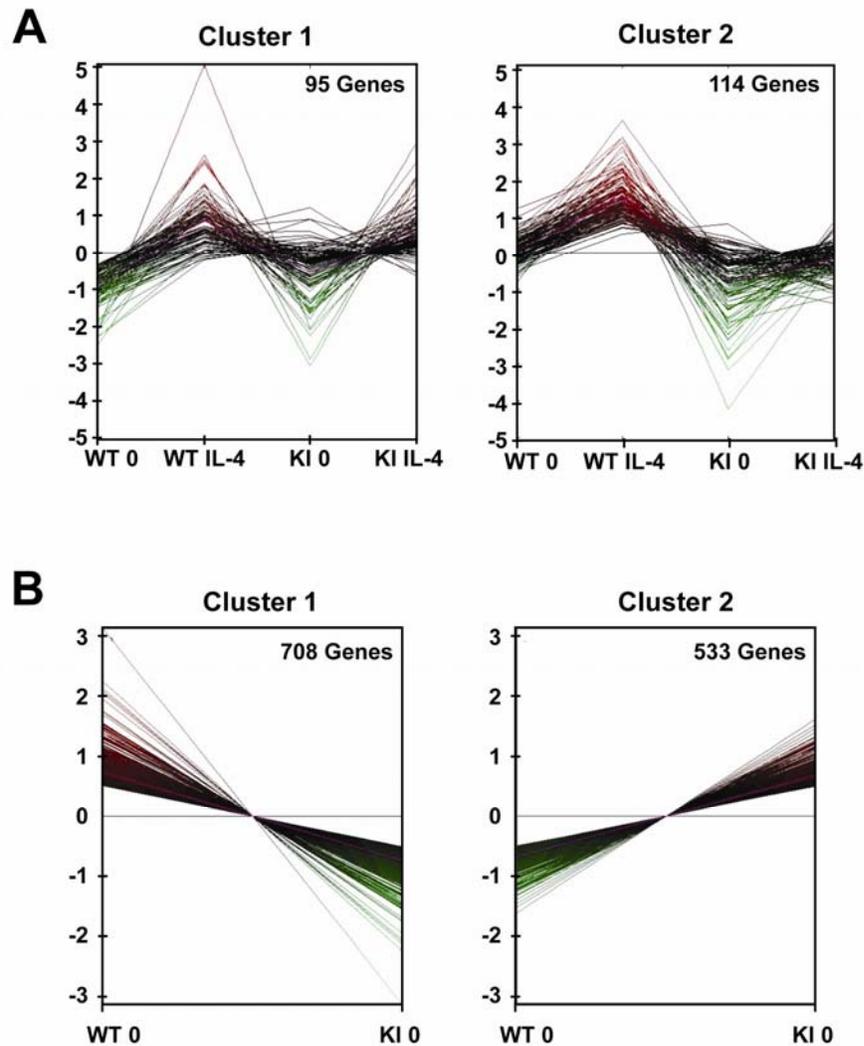


Fig. S4. Pangenomic transcription analysis reveals global changes in DAP12-KI macrophages. WT and DAP12-KI ThioM Φ were cultured in the presence or absence of IL-4 for 8 hrs. RNA was isolated and biotinylated antisense cRNA was hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 arrays. Expression values were background corrected, normalized, and summarized. Genes with changes by ≥ 2 -fold were included in the analysis. **(A)** Cluster analysis of genes upregulated after IL-4 treatment in WT macrophages. Cluster 1 shows genes expressed at similar levels in WT and DAP12-KI macrophages. Cluster 2 shows genes with lower expression levels in DAP12-KI macrophages. **(B)** Cluster analysis of genes differentially expressed in unstimulated WT and DAP12-KI macrophages. Cluster 1 includes genes expressed at lower, Cluster 2 genes expressed at higher levels in DAP12-KI macrophages when compared to WT. The entire data set and technical information requested by Minimum Information about a Microarray Experiment (MIAME) compliant are available at the Gene Expression Omnibus (GEO) website (www.ncbi.nlm.nih.gov/geo), accession number GSE13140.